Myosin head configurations in relaxed, active, rigor and S1-labelled fish muscle; Evidence for characteristically distinct states

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Introduction

Movement in all cells, whether they are muscle cells or not, is mediated by the action of motor proteins, such as myosin heads and kinesin, on filamentous 'tracks', respectively actin filaments (polymers of actin molecules) and microtubules (polymers of tubulin). In each case the motor protein is thought to attach to its track, to change its conformation in some way, thus producing movement, and then to detach again ready for another attachment - detachment cycle. In all cases the cycle is associated with the hydrolysis of ATP. Although a great deal has been learnt in the recent past about the atomic structures of some of these proteins [e.g. for muscle 5-7], it is still not clear exactly how movement is produced. Studies of intact muscle have the advantage that some muscles are extremely highly organised and they give rise to detailed X-ray diffraction patterns that, in principle, can be solved to yield details of the molecular organisation in the muscle. Muscle states can also be altered at will, so, for example, a muscle can be depleted of ATP, which normally promotes detachment of myosin heads from actin, so that long-lived attached states of myosin heads on actin are produced. Thus, although the microtubule motor systems can only be studied sensibly at tubule level in, for example, the electron microscope, the myosin/actin system can be studied by a variety of different structural methods which can even provide time-resolved structural details of the myosin motor in action [e.g. 1-4, 9-14].

Two particular muscle systems have been discovered that because of their superb degree of order provide technical advantages in structural studies. In the case of vertebrate muscles it is the muscles of fish that are particularly useful for this [1-4, 9-12], whereas in the case of the invertebrates it is insect flight muscles that are the most beautifully organised of all [15,16]. Figure 1(a) gives a generalised view of a muscle sarcomere, which is shown to comprise overlapping arrays of actin filaments and myosin filaments. The myosin heads (crossbridges) form projections on the myosin filament surface. In this position they can attach to, possibly 'swing' on, and then detach from neighbouring actin filaments in a typical contractile cycle. Figure 1(b) summarises the ATPase cycle associated with this mechanical crossbridge cycle. The work reported here concerns the myosin head/actin interactions in defined states of fish skeletal muscle (the fin muscles of plaice, Pleuronectes platessa) in order to provide a detailed insight into the myosin head cycle on actin in active muscle.

Relaxed Fish Muscle

The positions of the myosin heads in relaxed fish muscle have been determined by analysis of low-angle X-ray diffraction patterns (Figure 2) recorded on beamlines 16.1 and 2.1 at the CCLRC Daresbury Synchrotron Radiation Source [1-4]. Head shape was defined from the study ·of S1 crystals [5], with variable head configurational parameters given on each of the three different 3-fold symmetric 143Å-spaced 'crowns' of myosin heads within the 429Å axial repeat of the myosin filaments. Diffraction patterns were stripped using CCP13 fibre diffraction software [8] to give 56 independent intensity values out to a resolution of about 65Å. Searches and optimisation [3,4] were carried out using simulated annealing and local refinement procedures to give a 'best fit' structure with a crystallographic R-factor of about 4% (Figure 3(a)). The heads in this structure were all the same way up (i.e. with similar rotations around their own long axes), but they had a small range of axial tilts. The preferred structure also defined the orientation of the whole myosin filament array within the fish muscle A-band unit cell (Figure 3(b)). This positioned the myosin heads close to but not touching the adjacent actin filaments [3,4,9].
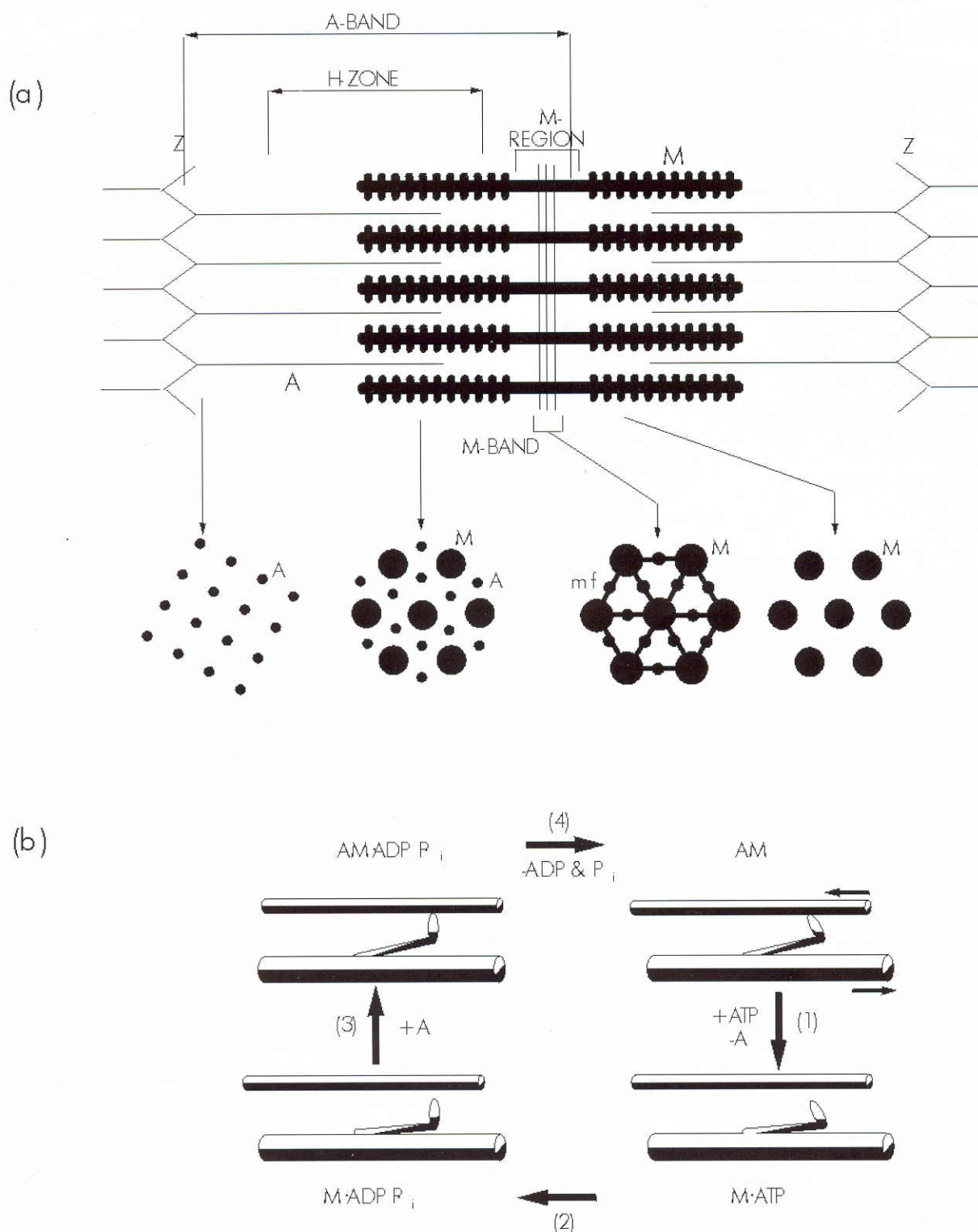
Figure 1: **(a)** Schematic diagram of a muscle sarcomere (Z to Z), showing the overlapping arrays of actin and myosin filaments. Sarcomere shortening occurs when these arrays slide past each other, powered by the action of myosin heads. **(b)** Correlation of a simplified mechanical cycle of myosin heads on actin and the corresponding stages in the hydrolysis of ATP [13].

Different Muscle States with Attached Myosin Heads

Head configurations are being determined for rigor and 'S1-decorated' rigor fish muscle (i.e. with no bound nucleotide - S1 are myosin heads proteolytically cleaved from the myosin molecule). This is being carried out by combined X-ray diffraction and electron microscopy studies of skinned muscle in rigor solution (Figure 2(c)), and in rigor solution, but also containing extrinsic myosin S1, in this case chicken papain S1 [9-11] (Figure 2(d)). Computed 3-D reconstructions (Figure 3(c)) of acto-S1 using X-ray amplitudes (Figure 2(c)) and phases from electron microscopy (courtesy R. Milligan) are informative and help to analyse the X-ray diffraction data that extend axially to about 10 Å resolution (see Harford *et al.* [11]).
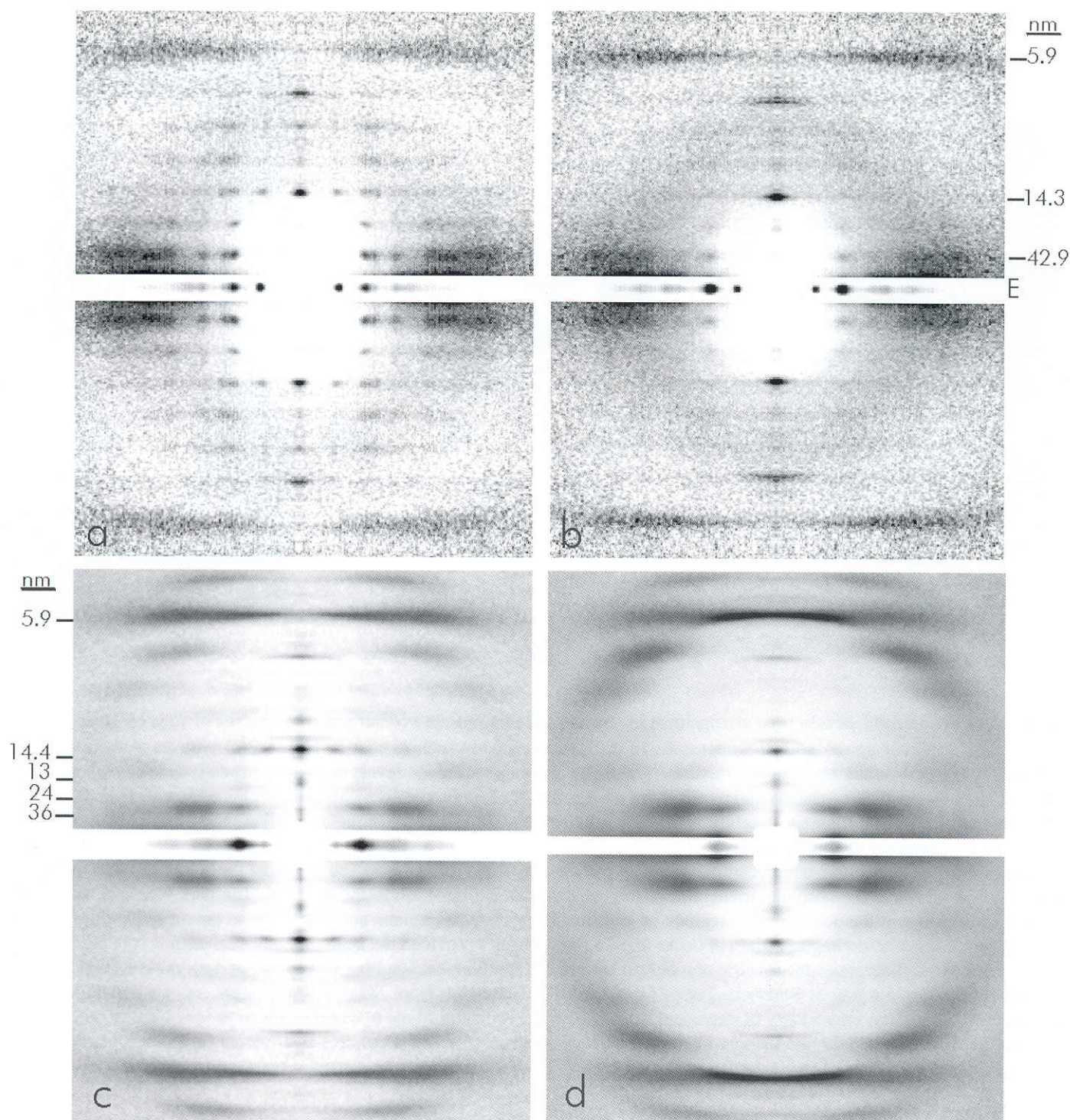
Figure 2: Low-angle X-ray diffraction patterns (fibre axis vertical) recorded from plaice fin muscles in a variety of static states. All patterns shown here were obtained at the CLRC Daresbury Synchrotron Radiation Source using beamlines 16.1 or 2.1 and using image plates or a 2D multiwire proportional detector. In all patterns the strong equatorial reflections (E) have been attenuated artificially by a horizontal strip. The intensities have been folded across quadrants and multiplied by the distance from the centre of the patterns to enhance the outer intensity features. Myosin layer-line orders of the 42.9 nm axial spacing are indicated on the right. **(a)** Relaxed muscle, **(b)** Fully active muscle - the steady state at the plateau of a tetanic contraction, **(c)** Skinned fish muscle in BDM rigor and **(d)** skinned rigor muscle as in (c) but labelled with exogenous myosin heads (S1). The lines in (c) indicate the characteristic 'rigor' layer-line intensities (orders of 72 nm) which are not expected or seen (e.g as in (d)) if the myosin heads label actin filaments uniformly on the actin filament helix. (c) is characteristic of a specific labelling pattern of myosin heads on actin in rigor muscle (Squire & Harford, 1988).

What is clear from a comparison of low-angle diffraction patterns from resting muscle (Figure 2(a)) and muscles with many heads bound to actin (Figures 2(c,d)) is that in the latter cases the actin layer-lines very clearly have enhanced intensity. However, the diffraction patterns from rigor muscle (Figure 2(c)) and from S1-labelled muscle (Figure 2(d)) are very clearly different. The S1-labelled
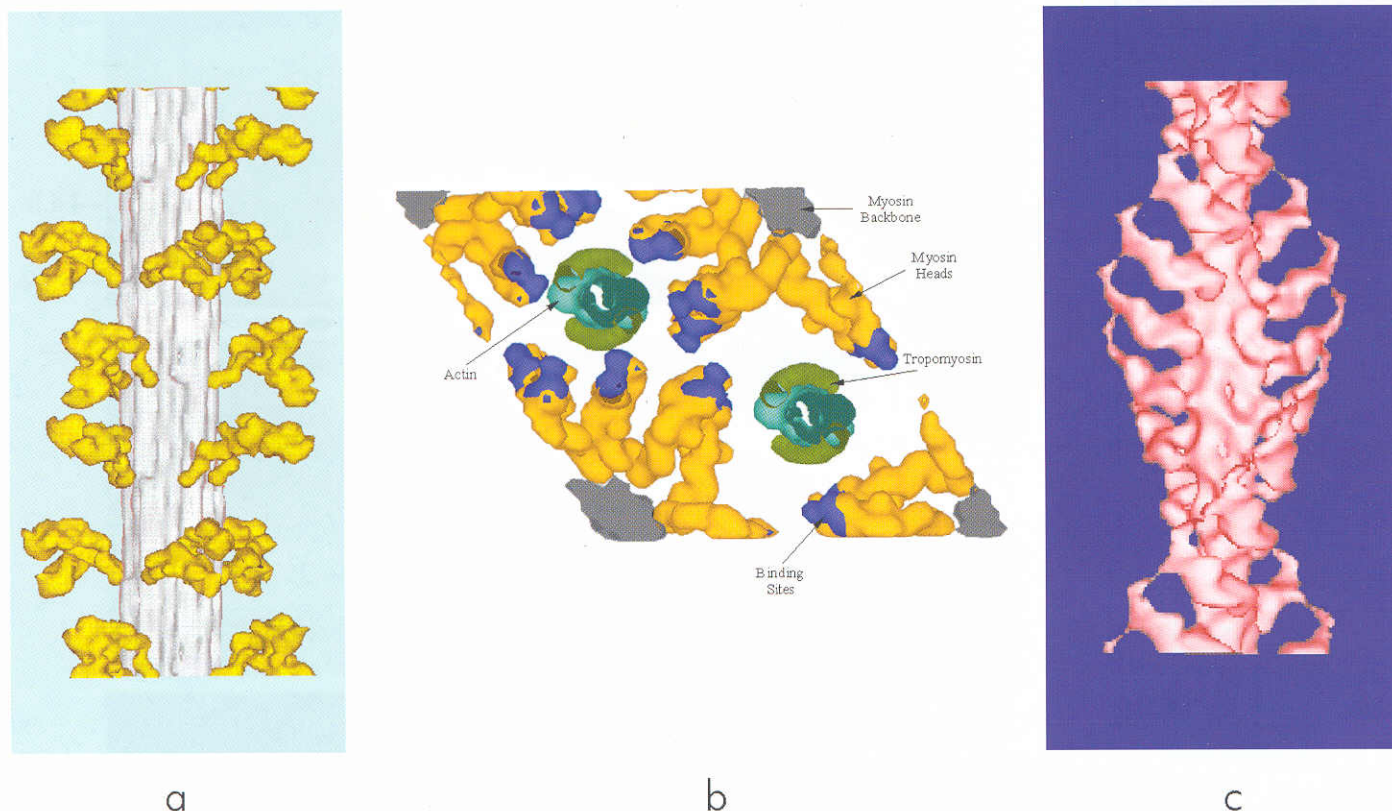
a

b

Myosin
Backbone

Myosin
Heads

Tropomyosin

Actin

Binding
Sites

c

Figure 3: (a) Optimised myosin head arrangement in resting plaice fin muscle as determined by Hudson *et al.* [11]. This structure (myosin heads yellow) agrees with the intensities in diffraction patterns such as that in Figure 2(a) with an R-factor of 3%; considerably better than any alternative structures. The figure shows about seven levels of myosin heads spaced at 14.3 nm axial intervals. The observed myosin head axial repeat is three times this at 42.9 nm. **(b)** Four unit cells of plaice fin muscle in the resting state including the myosin filament structure in (a). The grey patches at the corners of the unit cell are the myosin rod assemblies forming the backbones of the thick filaments. The myosin heads (yellow) are on the surface of these backbones. The green, ring-like structures represent actin-tropomyosin filaments in end-on view. The refinement used to model the X-ray diffraction data in Figure 2(a), also refined the absolute orientation of the myosin filament structure in the unit cell. The dark blue patches on the myosin heads represent the actin-binding regions of myosin. Many of these regions are very close to potential binding sites on actin, and stereospecific binding to actin will occur (e.g. in rigor muscle) provided the actin monomer azimuth is suitable for head attachment. It is this that creates the specific non-helical pattern of actin filament labelling that occurs in rigor muscle (Figure 2(c)). **(c)** 3-D helical reconstruction of S1-labelled actin using layer-line intensities from X-ray diffraction patterns such as Figure 2(d) and phases from electron microscopy of decorated actin filaments. The actin filament can be seen as the knobbly strand running up the centre of the figure with the tadpole-like myosin heads attached to it. The axial crossover repeat (near the top and bottom of the figure) is about 36 nm. See Harford *et al.* (1998).

muscle gives a pattern of intensities where the intensity peaks vary in radial position depending on which actin layer-line is involved. The observed diffraction pattern shows characteristic features of actin filaments helically-labelled with myosin heads, giving a helix-cross pattern of intensity. On the other hand rigor fish muscle gives layer-lines (Figure 2(c)) where the peak intensities are all at rather similar radial positions indicating more of a ladder of extra mass labelling the actin filaments. This is entirely consistent with previous analysis which has shown that rigor muscle has myosin heads organised into particular labelling patterns on actin; only azimuthally preferred actin monomers in 'target sites' are being labelled [17].

Myosin Heads with and without ADP

In the most recent study, we have tested whether there is any structural difference between (i) myosin heads in the rigor conformation with no bound nucleotide (Figure 4 left; cf Figure 2(c) which shows the inner part of a similar pattern) and (ii) in the biochemical state where the heads are labelled with ADP (Figure 4: right). Previous studies by electron microscopy and 3D reconstruction of actin filaments labelled with smooth muscle or non-muscle myosin heads [e.g. 18] have suggested that in those cases ADP produces a large conformational change in the myosin heads. However, judging from the results in Figure 4, there are only small structural differences
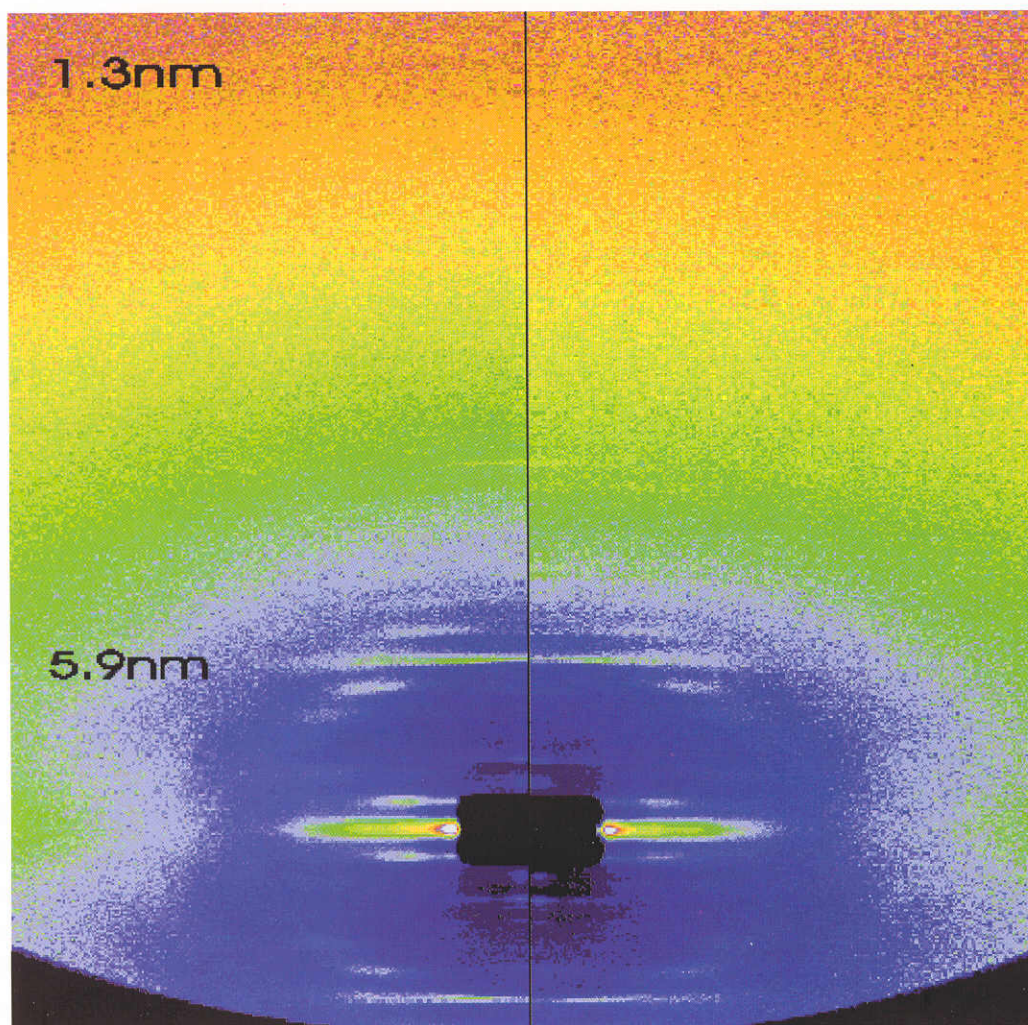
Figure 4: Diffraction patterns from skinned fish muscle in the rigor state showing actin layer-lines out to about 1.3 nm. The patterns were recorded using an image plate and 1.5 m camera length on line 16.1 at Daresbury. The right half of the pattern was from a muscle with ADP present, the left half from the same muscle without ADP. There appear to be only small structural differences between the two states.

between the skeletal muscle myosin head conformation in the presence and absence of ADP. The weak intensity of the layer lines at about 1nm possibly arises due to disordering of the myosin heads in the presence of ADP. This suggests that if any head shape changes occur in the contractile cycle of myosin heads on actin in vertebrate skeletal muscle, they are mainly on phosphate release between the states AM.ADP.Pi and AM.ADP (Figure 1(b)), rather than on ADP release between AM.ADP and AM (rigor). Previous time-resolved X-ray diffraction results on fish muscle have suggested the presence of two structurally different myosin head states on actin in active muscle [2,10,11]. These have been characterised as an initial non-force-producing (weak-binding) state, probably AM.ADP.Pi, and a subsequent force-producing (strong binding) state, probably a combination of AM.ADP and AM. Recent protein crystallography results on smooth muscle myosin [6] have suggested what the structures of two different myosin conformations may be.

Conclusions

The various diffraction patterns show clearly distinct intensity distributions: quasi-helical myosin head arrays on the myosin filament backbone in relaxed muscle (Figure 2(a)), quasi-helical arrays of myosin heads on actin in S1-labelled muscle (Figure 2(d)), head labelling of actin in distinct non-helical, ladder-like, patterns in rigor muscle (Figure 2(c)) and a mixed population of myosin heads on actin in active muscle (Figure 2(b)). There is only a small effect of ADP on the structure of skeletal muscle myosin heads on actin in rigor muscle. With the aid of CCP13 software and our own in-house modelling programs [4,8,10,11], these various states are being solved to define the changing myosin head arrangements between states and especially to determine the structural changes involved in force production and muscle shortening.

Acknowledgements

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Microphase separation in Poly(oxyethylene)-Poly(oxybutylene) Diblock copolymers

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Introduction

Diblock copolymers with narrow block length distributions may be readily prepared by sequential anionic polymerisation of ethylene oxide followed by 1,2-butylene oxide. We denote these copolymers E_mB_n, where E represents an oxyethylene unit $[OCH_2CH_2]$ and B an oxybutylene unit $[OCH_2CH(CH_2CH_3)]$. Their bulk properties are of significant interest, since microphase-separated structures may form from the disordered melt when the temperature is lowered, either by crystallisation of the E blocks[1-3], or by microphase separation in the melt state[4].

Experimental Procedure

Diblock copolymers were prepared by sequential anionic polymerisation of ethylene oxide (EO) followed by 1,2-butylene oxide (BO). Details of the methods have been published[4]. Characterisation of the intermediate poly(oxyethylene) and the final copolymer was by gel permeation chromatography (GPC) and ^{13}C NMR spectroscopy.

Time Resolved Small-Angle X-Ray Scattering

SAXS measurements were carried out on Beamline 8.2 of the SRS, Daresbury Laboratory, Warrington, UK[6]. The loaded pans were placed in the cell of a Linkam DSC of single-pan design. Samples were heated from room temperature to T_{ODT} + 30 °C at 10 °C min^{-1}, held at the maximum temperature for 1 min